Plug ‘n’ Play with DNA

Lund, Anne Mathilde; Andreasen, Elisa W.; Korgaard, Jaide V.; Rank, Julie; Knudsen, Maja Skovbjerg

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Team DTU Denmark 2

Anne Mathilde Lund, Elisa W. Andreasen, Jaide V. Korgaard, Julie Rank, Maja Skovbjerg
Supervisors: Mikael Rørdam Andersen, Helene Faustrup Kildegaard, Dorte Koefoed Holm
Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark

A Novel Assembly System

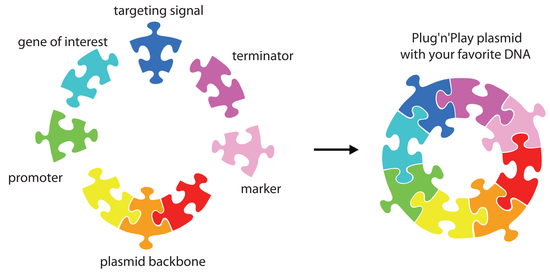
Synthetic biology has evolved dramatically within the past decade, which calls for a revolution of the Standard Assembly method that makes the foundation of BioBricks. We believe that iGEM should be about fast assembly of BioBricks, where any thinkable part, device or existing BioBrick can be combined for any type of organism within one day. Therefore, we have designed a new BioBrick Kit based on a novel assembly standard; called “Plug 'n' Play with DNA”.

Achievements

- Development of a standardized assembly system
- Development of a guide for customization
- Construction of 49 biological parts and more than 21 unique devices
- Proof of concept for U-2 OS cell line and *Aspergillus nidulans*
- Characterization of two fungal promoters
- We have helped another iGEM team by generating biological parts for bacteria

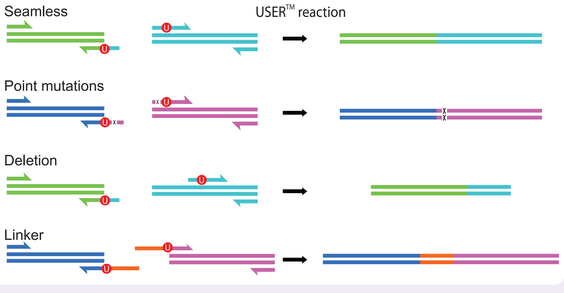
Plug ’n’ Play with DNA

The new assembly standard makes it possible to gather any biological parts without use of restriction enzymes and ligases. The USER cloning enables numerous biological parts in the form of pre-produced PCR-products to form a ready to use vector in just one reaction.

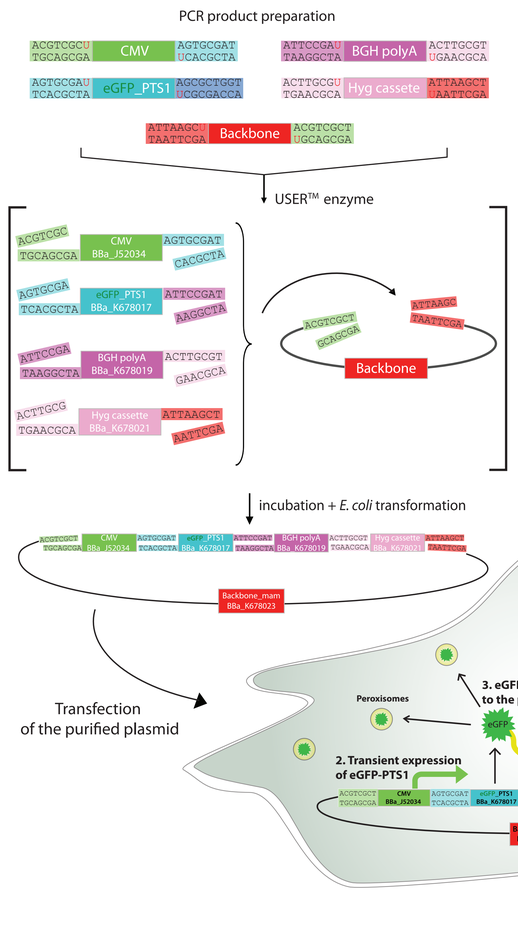


Tailoring the System

Standardizing a technology entails rigidity. However, the Plug ‘n’ Play assembly standard is based on the USER cloning technology, which also enables customization.This makes it easy to introduce specific modifications such as seamless assembly, introducing point mutations or extra linkers [1,2].



Cloning Strategy

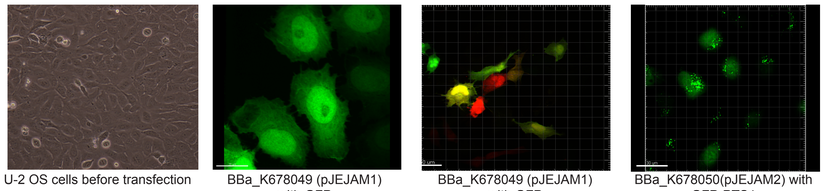


The USER cloning is a ligation independent cloning technique. The method applies long complementary overhangs to anneal to each other to form a stable hybridisation product that can be used to transform E. coli without prior ligation. The overhangs on the PCR products are custom-made and their generation is restriction site-independent. Assembly of complex DNA constructs made of several fragments can easily be fused seamlessly together [3,4]. By using transient transfection, mammalian cell line U-2 OS is transfected with a plasmid able to express GFP with a targeting sequence localizing the produced GFP to the peroxisomes of the cell.

Fluorescence Reporter System

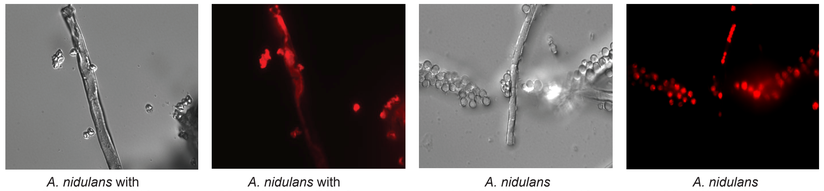
As proof of concept we have created a reporter system that can be used for anything from monitoring gene expression to determination of protein localization. Within 2 months we constructed 10 plasmids with our Plug ’n’ Play assembly standard. The different fluorescent proteins were expressed in the model organism *Aspergillus nidulans* as well as in the mammalian cell line U-2 OS with success.

Mammalian cells



U-2 OS cells before transfection | BBa_K678049 (pJEJAM1) with GFP | BBa_K678049 (pJEJAM1) with GFP BBa_K678052 (pJEJAM4) with mCherry | BBa_K678050(pJEJAM2) with GFP-PTS1

Fungi



A. nidulans with BBa_K678062 - DIC | *A. nidulans* with BBa_K678062 - RFP filter | *A. nidulans* with BBa_K678063 - DIC | *A. nidulans* with BBa_K678063 - RFP filter

Acknowledgement

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References

1. Nørholm, M.H.H. A mutant Pfu DNA polymerase designed for advanced uracil-excision DNA engineering. BMC Biotechnol. 10, 21, 2010.
2. Figure inspired from Genee HJ, Bonde MT, Bagger FO, Olsen LR. PHUSER v2 (In preparation).
3. Hansen B.G. et al. Versatile Enzyme Expression and Characterization System for *Aspergillus nidulans*, with the *Penicillium brevicompactum* Polyketide Synthase Gene from the Mycophenolic Acid Gene Cluster as a Test Case Appl Environ Microbiol. 2011 May;77(9):3044-51.
4. Nour-Eldin, H.H. et al. USER cloning and USER fusion: the ideal cloning techniques for small and big labo ratories. Methods Mol Biol. 2010;643:185-200.